

RESEARCH ARTICLE

Antifertility Effect of the Aqueous, Alcoholic and Petroleum Ether Extract of *Cascabella thevetia* (L.) Fruit in Female Albino RatsVandana Deshmukh¹, Varsha Zade^{*2}¹Vandana Deshmukh, Research Scholar, Government Vidarbha Institute of Sciences and Humanities, Amravati-444 604 (MH), India²Varsha S Zade, Associate Professor, Government Vidarbha Institute of Sciences and Humanities, Amravati-444 604 (MH), India

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ABSTRACT

The present work deals with antifertility effect of the aqueous, alcoholic and petroleum ether extract of *Cascabella thevetia* (L.) fruit in female albino rats. Rats weighing 120 to 200 gm were randomized into 4 groups (A to D) and laprotomised on 10th day of pregnancy and the live fetuses were observed in both the horns of the uterus. Rats in group A (control) were orally administered, once daily with 0.5 ml of distilled water while those in group B to D served as experimental groups and were administered aqueous, alcoholic and petroleum ether extract of *Cascabella thevetia* (L.) fruit (100, 200 and 400 mg/kg body weight doses). The extract of *Cascabella thevetia* (L.) fruit exhibited significant post coital antifertility activity. It was found that the extract significantly reduced the number of live fetuses, whereas the resorption index and post implantation losses increased significantly. The % of abortion was found to be highest (100%) with 200 mg/kg dose of aqueous extract of *Cascabella thevetia* (L.) fruit. In the present study it was observed that the aqueous, alcoholic and petroleum ether extract of *Cascabella thevetia* (L.) fruit extract at 200, 400 and 400 mg/kg body weight prolonged the estrous cycle and particularly diestrus phase in the experimental animals. The phytochemical screening of *Cascabella thevetia* (L.) fruit revealed the presence of alkaloids, steroids, flavonoids and tannins whereas simple phenolics, and saponins and anthroquenon are absent.

Key words: Antifertility activity, *Cascabella thevetia* (L.) fruit, Estrous cycle, Hormonal level Female albino rat.

INTRODUCTION

Plant materials continue to play an important role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs originated from natural plant sources [1]. These plant products are the major source of drug development in pharmaceutical industry [2]. Antifertility is a term used for the prevention of pregnancy, and it is often referred to as birth control. The basic aim of antifertility drugs is to prevent conception or fertilization. There are drugs that control ovulation and if regularly consumed, function as effective contraceptives. Oral contraceptives belong to the class of natural products known as steroids. These control the female menstrual cycle and ovulation.

The scientists all over the world are putting much emphasis on herbs and plants in the fertility control. Herbal drugs are usually effective, inexpensive & afforded by people of rural area. [3-7]

In the present work antifertility efficacy of fruit of the plant *Cascabella thevetia* (L.) were evaluated. *Cascabela thevetia* (L.) is a small evergreen plants species of shrub or small tree belonging to the family Apocynaceae. Common names are Be-still tree, Captain Cook tree, dicky plant, foreigner's tree, lucky nut, Mexican oleander, still tree, yellow oleander, Currant-tree. It is a large spreading shrub usually 2.5-3.5m tall.

- *Thevetia peruviana* (*Cascabela thevetia*) is used medicinally throughout the tropics in spite of its toxicity.
- A bark or leaf decoction is taken to loosen the bowels, as an emetic, and is said to be an effective cure for intermittent fevers.
- In Senegal, water in which leaves and bark were macerated is taken to cure amenorrhoea.
- In Mali, the latex is applied to soften corns and calluses.
- In Côte d'Ivoire and Benin, the leaf sap is used as eye drops and nose drops to cure violent headaches; the leaf sap is also dropped in the nostrils to revive people that have fainted and to cure colds.
- In Kenya, the Luo people use water in which leaves have been crushed to treat colds.
- The seeds may be used as a purgative.
- The seed oil is applied externally in India to treat skin infections.
- In Ghana, the leaves in decoction are taken to treat jaundice, fever and as a purgative for intestinal worms.^[8]

MATERIALS AND METHODS

Collection of plant material

The plant *Cascabella thevetia* (L.) fruit was collected from Amravati region and identified and authenticated by experts from Botanical survey of India, Pune where, a voucher specimen of the plant has been deposited in the herbarium of the department. (Accession No. VADCAP-2)

Procurement and rearing of experimental animal

Albino rats (Wistar strain) used in the present investigation were procured from S.N. Institute of Pharmacy, Pusad (M.S.). The rats were acclimatized for 15 days to the best laboratory condition (prior to experiment) and maintained on balanced diet (Trimurti lab feeds, Nagpur). Water was provided *ad libitum*. All procedures with animals were conducted strictly in accordance with approved guideline regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, Ministry of Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering. All experimental protocols were met with the approval of Institutional Animal Ethics Committee

registration number 1060/ac/07/CPCSEA (IAEC/7/2009).

Preparation of extract

The fruit of *Cascabella thevetia* (L.) were collected, shade dried, powdered and subjected to soxhlet extraction successively with aqueous, alcoholic and ether extract. The extract was evaporated to near dryness on a water bath, weighed and kept at in 4 °C refrigerator until the experimental testing.

Phytochemical screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as described by Thimmaiah.^[9]

Acute toxicity study

The animals were divided into three groups and the extract was administered orally at the doses of 1000, 2000 and 4000 mg/kg body weight separately. Control rats received the vehicle only. The rats were observed for 72 hr. for behavioral changes and mortality^[10].

Antifertility testing

The plant extract of *Cascabella thevetia* (L.) fruit were tested in female albino rats for abortifacient activity by the method by Khanna and Chaudhary^[11]. The vaginal smears of caged female rats of known fertility were monitored daily. Unstained material was observed under a light microscope. The proportion among the cells observed was used for determination of the estrous cycle phases^[12]. The female rats were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as 1st day of pregnancy. These rats were randomly distributed into 4 groups, a control group and three experimental groups of 6 animals each. On the day on the 10th day of pregnancy animals were laprotomised under light ether anesthesia using sterile condition. The two horns of uteri were examined to determine the implantation sites. There after the abdominal wound was sutured in layers. Post operational care was taken to avoid any infection. The extract to be tested were then fed to operated pregnant rats i.e. aqueous, alcoholic and ether extract of *Cascabella thevetia* (L.) fruit at doses 100 mg/kg, 200 mg/kg, 400 mg/kg of each extract specified by an intragastric (i.g.) soft rubber catheter from day 11

up to the day15 of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the antifertility activity of extract was evaluated. Litters were examined for any malformations.

Effect on estrous cycle

The aqueous, alcoholic and ether extract of *Cascabella thevetia* (L.) fruit at a dose of 200,400 and 400 mg/kg body weight respectively was found to be active amongst the three treatments in antifertility testing. Hence it was subjected to a detailed investigation for study of estrous cycle. The studies were conducted on adult female rats (140-180 gm) for 30 days. To study the estrous cycle pattern, animal showing regularity in the normal cycle were separated and chosen for further studies. Those animals showing normal estrus cycle were divided in two groups of 6 animals each; Group I- control, received distilled water (Vehicle) and Group II-III and IV treated with aqueous, alcoholic and ether alcoholic extract at dose of 200,400 and 400 mg/kg body weight respectively. Vaginal smear using saline solution were taken twice daily during the entire treatment period, observation of the vaginal opening and the cell type obtained in a vaginal smear was also done. The duration of estrous cycle together with that of various phases was determined [13].

Effect on hormonal level

The aqueous, alcoholic and ether extract of *Cascabella thevetia* (L.) fruit at a dose of 200,400 and 400 mg/kg body weight respectively, was

found to be most active treatments in the abortifacient testing. Hence rats treated with aqueous, alcoholic and ether extract were subjected to a detailed investigation for the study of hormonal assay. Sexually experienced female albino rats were divided into 4 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2- 4 received suspension of the aqueous, alcoholic and ether extract of *Cascabella thevetia* (L.) fruit orally at the dose 200, 400 and 400 mg/kg, daily for 30 days. After 30 days of treatment all the control and experimental groups of female rats sera were analyzed for estrogen, progesterone, luteinizing and follicle stimulating hormone level with AccuLite master CLIA VAST Enabled kit by Chemiluminescence immunoassay (CLIA) method with semi automated Chemiluminescence analyzer and autoplex- A processor for CLIA. [14- 15]

Hormonal assay

The animals were anesthetized with ether; blood samples were collected directly from their hearts and centrifuged at 2000-3000 rpm for 15 minutes at 4°C. Serum samples were stored at -20°C until assayed for FSH. Serum concentrations of FSH were measured by the chemiluminescence immunoassay (CLIA) method.

Statistical analysis

All the data are expressed as mean ± SEM. Statistical analysis was done by using paired and unpaired student's t-test [16].

RESULTS

Preliminary Phytochemical investigation

Table 1: The phytochemical screening of different extracts of *Cascabella thevetia* (L.) fruit revealed the presence of various constituents as shown in table

Name of the plant	Alkaloids	Anthraquinone	Flavonoids	Simple phenolics	Steroids	Tannins	Saponins
<i>Cascabella thevetia</i> (L.) fruit	+	-	+	-	+	+	-

Present +, Absent -

Acute toxicity studies

No mortality and changes in the behaviour was observed in the treatment groups up to 4000 mg/kg body weight and from the results 400 mg/kg dose was chosen as maximum dose for further experimentation

Abortifacient activity

The oral administration of *Cascabella thevetia* (L.) fruit extract (aqueous, alcohol, ether) at the

doses of 100, and 200 mg/kg and 100,200,400mg/kg and 100, 200,and 400 mg/ kg body weight produced respectively a dose dependent adverse effect on fertility index and on number of implantation in the uterine horns of the female rats by virtue of an increase in the percentage of the post-implantation embryonic loss. All the experimental extracts when evaluated for their abortifacient activity, were found to exhibit pregnancy interceptive activity.

Administration of 100 and 200 mg/kg body weight of the aqueous extract resulted in 58.13% and 100% abortion (Table 1). Administration of 100 and 200 and 400mg/kg body weight of the alcoholic extract resulted in 25%,45.65 and 70% abortion Administration of 100, 200 and 400 mg/kg body weight of the ether extract resulted in 39.02%,69.09% and 89.28% abortion This was evident from decrease in the percentage of live fetuses and fetus abortion respectively. The percent resorption index increased from zero in the control animals to 100 % in 200 mg/kg body weight in aqueous extract treated animals, 70% in 400 mg/kg body weight in alcoholic extract treated animals. 89.28% in 400 mg/kg body weight in ether extract treated animals There was a decrease in litter size with increase in the dose of the plant *Cascabella thevetia* (L.) fruit extract in all the treatment groups. The litter size of

control group rats was the highest (6.86±0.50). The litter body weight recorded in animals administered with alcoholic, aqueous and ethyl acetate extract of the *Cascabella thevetia* (L.) fruit were not significantly different from control. Similarly, the total body length of litters at day 1 of birth also did not vary significantly from that of control. When the sex ratios of litter were determined it was found that the male sex was dominant to female sex. The gestation period did not show any variation in extract treated group of animals as compared to control group (Table 2). Further, the dose dependent increase in the resorption index due to the administration of the extract, in the present study is an indication of failure in the development of the embryo. Such occurrences of foetal resorption suggest that interruption of pregnancy occurred after implantation of the foetus [17].

Table 2: The effect of aqueous, alcoholic, ether extract of *Cascabella thevetia* (L.) fruit on fertility of rats when fed orally from day 11 to 15 of pregnancy

Treatment groups (dose, mg/kg body wt)	Sample size	No. of foetus observed in individual rats on day 10	No. of rats delivered (litter size)	No. of resorption in individual rats	No. of resorption (mean±SE)	Abortifacient activity (%)	
Control	Vehicle	6	6(10,8,8,8,9,12)	6(10,9,8,8,9,12)	0,0,0,0,0,0	Nil	
Aqueous Extract	100	6	6(4,6,8,7,9,9)	6(1,3,4,3,3,4)	3,3,4,4,5,6	4.16±0.47	58.13%
	200	6	6(6,9,8,6,10,7)	6(0,0,0,0,0,0)	6,9,8,6,10,7	7.66±0.66	100%
Alcoholic extract	100	6	6(10,8,8,7,6,9)	6(8,8,6,6,4,4)	2,0,2,1,2,5	2+0.68***	25%
	200	6	6(7,7,8,6,9,9)	6(4,3,4,3,5,6)	4,3,4,3,4,3	3.5±0.22	45.65%
	400	6	6(10,12,11,9,8,10)	6(4,4,3,3,3,1)	6,8,8,6,5,9	7±0.63***	70%
Petroleum ether extract	100	6	6(7,6,6,5,9,8)	6(5,4,4,2,4,6)	2,2,2,3,5,2	2.66± 0.49	39.02%
	200	6	6(10,12,9,9,8,7)	6(3,3,1,2,0,2)	7,3,8,7,8,5	6.33±0.80**	69.09%
	400	6	6(7,10,11,8,8,12)	6(1,2,1,0,0,2)	6,8,10,8,8,10	8.33± 0.61	89.28 %

Values are expressed as mean ± S.E. for six albino rats in each group . P values: *<0.05, **<0.01, ***<0.001, When compared between group, ns= non significant

Table 3: Effect of aqueous, alcoholic, ether extract of *Cascabella thevetia* (L.) fruit on fertility of female albino rats when feed orally from day 11 -15 of pregnancy

Parameters								
Treatment Groups		Gestation period (days)	Litter size (No.)	Litter body Weight (gm)	Total body length of litter at 1 st day of birth (mm)	Sex ratio of live fetuses (Male/female)	% Viable fetuses	% Fetuses resorptions
Control Group-I (Vehicle)		22.64±0.01	6.86±0.50	5.73.46±0.06	59.9 ±0.01	24/21	100	0
Aqueous Extract of of <i>Cascabella thevetia</i> (L.) fruit	Group-II 100 mg/kg B.W	22.20±0.01*	6.64±0.40*	5.07±0.01	34.68.0±0.05	19/18	42.87	58.13
	Group-II 200 mg/kg B.W	0	0	0	0	0	0	100
Aqueous Extract of of <i>Cascabella thevetia</i> (L.) fruit	Group-II 100 mg/kg B.W	22.35±0.39***	6.34±0.01	4.85±0.01*	56.5±0.01**	20/17	75	25
	Group-III 200 mg/kg B.W	22.75±0.01***	6.51±0.01*	4.76±0.01***	54.6±0.01*	13/12	55.45	45.65
	Group-IV 400 mg/kg B.W	22.44±0.01**	6.74±0.01	4.45±0.01**	55.5±0.01*	15/16	30	70
Aqueous Extract of <i>Cascabella thevetia</i> (L.) fruit	Group-II 100 mg/kg B.W	22.85±0.01**	6.69±0.01	4.30±0.01**	57.20±0.01	22/20	61.98	39.02
	Group-III 200 mg/kg B.W	22.75±0.01*	6.49±0.01**	4.83±0.01***	53.8±0.01**	12/10	31.91	69.09
	Group-IV 400 mg/kg B.W	22.53±0.01***	6.25±0.01*	4.54±0.01*	51.10±0.01*	24/22	11.72	89.28

Values are expressed as mean ± S.E. for six albino rats in each group. P values: *<0.05, **<0.01, ***<0.001, When compared with control, ns= non significant

Effect of extracts of *Cascabella thevetia* (L.) on the estrous cycle of rats

Administration of 200 mg/kg,400mg/kg, 400mg/kg respectively, aqueous, alcoholic & petroleum ether extract of seed extract of

Cascabella thevetia caused an irregular estrous cycle (Table 4), with an increase in the diestrous phase while the proestrous and metaestrous phase were found to be significantly reduced as compared to the control. Withdrawal of the

treatment did not indicate any significant change either in the four phases of the estrous cycle or in the duration of the cycle.

Table 4: Effect on estrous cycle of female albino rats after the administration of 400 mg/kg aqueous, alcoholic, and 200 mg/kg ether extract of *Cascabella thevetia* (L.) fruit

Phases	Proestrous phase (days)	Estrous phase (days)	Metaestrous phase (days)	Diestrous phase (days)	Estrous cycle (days)
Vaginal opening/ cell type obtained in a vaginal smear	25% to 40% / Epithelial cells only	Above 70% / Few cornified cells	50% to 70% / Cornified cells plus many leukocyte	50% to 70% / Leukocytes plus epithelial cells	
Group- A- Control	0.59±0.01	0.65±0.01	0.59±0.01	0.65±0.01	4.42±0.68
Group-B Aqueous Extrac200mg/kg	0.55± 0.03**	0.54± 0.08***	0.76± 0.07*	3.05± 0.43***	4.78± 0.02**
Group-C alcoholic Extract 400 mg/kg	0.46± 0.07***	0.56± 0.01***	0.79± 0.01**	2.49± 0.02***	4.30± 0.09***
Group-D Petroleum Extract 400mg/kg	0.44± 0.06*	0.50± 0.02**	0.74± 0.01	3.09± 1.51***	5.06± 0.81***

Values are expressed as Mean ± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***p<0.001, when compared with control.

Hormonal assay

In the present study after the administration of aqueous, alcoholic, and ether extract of *Cascabella thevetia* (L.) fruit at dose of 200mg/kg , 400mg/kg and 400mg/kg body weight respectively, significant alteration was accounted in the concentration of steroid hormones such as serum estrogen, progesterone, LH, FSH . Administration of rats with aqueous, alcoholic and ether extract of *Cascabella thevetia* (L.) fruit at a dose of 200mg/kg , 400mg/kg and 400mg/kg body weight

respectively showed a reduction in significant level of LH and FSH as compared to control rat. On the other hand, it was observed that the level of estrogen increased and there was a slight decrease in the progesterone hormone in the extract treated animals (Table 5). This results suggest that high dose of estrogen disproportionate to progesterone leads to resorption of fetuses , this effect may due to the imbalance of estrogen and progesterone level.

Table 5: Effect of aqueous, alcoholic, ether extract of *Cascabella thevetia* (L.) fruit at a dose of 200,400,400 mg/kg body weight on hormone levels of female albino rat

Treatment Groups	Dose mg/kg	FSH(pg/ml)	LH(pg/ml)	Estrogen(pg/ml)	Progesterone(pg/ml)
Group I-Control	Vehicle	1.93 ± 0.05	0.41 ± 0.05	78.42 ± 0.06	57.33 ± 0.04
Aqueous extract of <i>Cascabella thevetia</i> (L.) fruit	200mg/kg	1.66 ± 0.01***	0.35 ± 0.01**	80.29 ± 0.03***	53.6 8 ± 1.71*
Group I-Alcoholic extract of <i>Cascabella thevetia</i> (L.)	400mg/kg	1.4 5 ± 0.01***	0.28 ± 0.02**	80 ± 0.02***	49.42 ± 0.03****
Ether extract of <i>Cascabella thevetia</i> (L.)	400mg/kg	1.33 ± 0.02***	0.28 ± 0.41*	87.24 ± 0.01***	43.80 ± 0.02***

Values are expressed as *<0.05, **<0.01, ***<0.001, When compared with control, ns= non significant

DISCUSSION

Flavonoids have been reported to possess antifertility activity [18, 19, 20, 21, 22]. In the present study the antifertility activity of the extract may be due to the presence of flavonoids or other constituent in it. According to the literature, flavonoids and saponins are known to exhibit antifertility activity. [23, 24, 25] The *Cascabella thevetia* (L.) fruit extract displayed antifertility significant activity when compared with controls, indicating that flavonoids could be responsible for the activity.

It is well known fact that estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing hormone (LH), which in turn prevent the implantation. Estrogen and progesterone are the hormones responsible for histology and functional modifications of female genital tract. [26]

The female reproductive activity is under the combined and balanced influences of ovarian and extra ovarian hormones. [27] Imbalances or alterations in these hormones lead to irregularity in the reproductive functions. Previous studies show that the hormonal imbalances are caused by numerous chemical agents contained in plant extracts. [28,29] The aqueous extract of *Cascabella thevetia* (L.) fruit howing to its estrogenic nature may have altered the biochemical milieu of the uterus which lead to a change in the normal status of reproduction in female reproductive tract of rats and thus produced significant antifertility activity. Our findings agree with that [30,31] on administration of *Balanites roxburghii* extract and *Hibiscus rosasinesis* root extract respectively on immature ovariectomized rats.. Present work indicates that the administration of the extracts of *Cascabella thevetia* (L.) fruit showed significant

increase in the estrogen level which might induce an inhibitory effect on gonadotropins. The variations observed in the reproductive organ weights in the treated rats might be attributed to phytoestrogenic components of the extract.

Alcoholic extract of *Neem* flowers alters the estrous cycle, by prolonging the duration of the diestrus phase and subsequently lowering the frequency at which the estrus phase occurs. Consequently the frequency of ovulation is reduced and fertility may therefore be impaired.^[32,33,34,35,36] In the present study of aqueous, alcoholic and ether extract of a *Cascabella thevetia* (L.) fruit 400, 400 and 100 mg/kg body weight respectively, shows the antifertility effect of prolongation of estrous cycle and diestrus phase particularly in experimental animals. The results of our study (Table 1) confirm the reports of the ability of some plant extracts to prolong the oestrus cycle and diestrus phase of the cycle^[37,38]. In this study, the three extracts induced significant increase in the estrous phase duration and decrease of metestrous and diestrous phases.

CONCLUSION

The results of the present study indicate that the extract of *Cascabella thevetia* (L.) fruit have significant antifertility activity. The antifertility effect of aqueous, alcoholic and ether extract of *Cascabella thevetia* (L.) fruit appears to be possibly due to its anti estrogenic effect, either by blocking the estrogen receptors or by diminished estrogen synthesis. The extract of *Cascabella thevetia* (L.) fruit of this plant could be used to induce abortion and can further be developed into a contraceptive.

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